Probiotic use in equine gastrointestinal disease

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Abstract: Probiotics are commonly used in human and veterinary medicine due to their postulated positive effects on overall and specifically gastrointestinal health. Although some beneficial effects have been shown in several human diseases, a general beneficial effect of probiotics is currently not supported. In horses, well-designed studies to date are few, results are conflicting, and the effects of probiotics are questionable. Adverse effects are rare; however, intestinal adverse effects (diarrhea) have been reported in foals. Quality control of over-the-counter probiotics is not tightly regulated, and labels often do not reflect the content.

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Probiotic Use in Equine Gastrointestinal Disease

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KEYWORDS

• *Lactobacillus* • *Bifidobacterium* • *Saccharomyces boulardii* • Microbiota
• Fecal microbial transplantation

KEY POINTS

- Mechanisms of action include modulation of the immune system, antimicrobial production, bacterial toxin inactivation, and an increase in colonization resistance.
- Probiotics are generally considered safe, and adverse effects are rare; however, adverse effects have been reported in foals and therefore should be used with caution.
- The quality control of commercial human and veterinary probiotics products is poor and the content of over-the-counter probiotics is often inaccurate regarding bacterial species and amount of live organisms contained in a product.
- The evidence behind efficacy of probiotics in equine gastrointestinal disease is weak and their beneficial effects are questionable.
- Future research on the use of probiotics should focus on using different strains, such as members of families with high abundance in the gastrointestinal system of horses, or a mix of many bacterial strains, similar to fecal microbial transplantation.

INTRODUCTION

Elie Metchnikoff[1] studied the longevity of a group of Bulgarians in the 1900s. He observed that these people ate large amounts of fermented milk and postulated that the bacteria responsible for fermentation had a positive effect on the health of the consumers. These bacteria were named *Lactobacillus bulgaricu*s and the idea of probiotics was born. Metchnikoff initially defined probiotics as “live microorganisms, which exhibit a health promoting effect” in 1908.[1] Although research initially flourished and more probiotic bacterial strains were discovered, it then drifted to the fringe of medical practice and was rediscovered in the mid-1990s. The idea of probiotics exerting beneficial effects has since re-entered the humane medical field and achieved mainstream medical interest. The Food and Agricultural Organization and World Health Organization modified the initial definition to “live microorganisms,

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that when administered orally at adequate concentrations, provide a beneficial effect beyond that of their nutritional value."[2]

GENERAL CONSIDERATIONS

Information regarding probiotics is accumulating rapidly and is widely available on the Internet and from various sources; thus, it is important to understand some practical aspects regarding formulation and labeling of probiotics that make it challenging to provide direct comparisons and interpret results of studies.

Several microorganisms, including yeasts and bacteria are used as probiotics (**Box 1**). Some bacterial families, mainly lactic acid producers, such as lactobacilli and bifidobacteria, are commonly used. Not all members of the same family have probiotic properties; for example, not all lactobacilli are suitable for probiotic use. Lactobacilli per se are often called probiotics or used as such, but not all lactobacilli have probiotic properties; therefore, the administration of commercial yoghurts is unlikely to be of benefit to the horse. Potential probiotic strains need to be evaluated for suitability of their probiotic characteristics.[3] Potential probiotic strains should be able to survive the gastric environment, have antimicrobial properties, and adhere to mucus and epithelial cells.[2] Not all strains survive the extrusion and drying, which leads to low numbers of active colonies in the end product. The manufacturing process affects the ability of bacteria to maintain desirable traits. Manufacturing a probiotic strain under different conditions, such as varying culture media or coculturing with prebiotics or other probiotic strains, leads to expression of different traits and differences in the final product.[4,5] It is crucial to remember, when comparing results of commercial probiotic

Box 1
Bacterial genera and yeasts typically used as probiotics

Saccharomyces (yeast)[a]
Lactobacillus[a]
Bacteroides
Escherichia coli
Enterococcus[a]
Bacillus[a]
Nitrobacter
Nitrosomonas
Streptococcus[a]
Rhodobacter
Fusobacterium
Butyrivibrio
Rhodobacter
Clostridium
Eubacterium
Bifidobacterium[a]

Genera are bacteria unless stated otherwise.
[a] Evaluated as probiotics in horses.

product studies, that manufacturing methods matter and products containing the same strain at the same concentration cannot be directly compared.

Most diseases of the gastrointestinal tract in horses affect the large intestine. Accordingly, a probiotic used for horses should ideally exert effects in the cecum and colon of horses. Recent advances in research have shown that the most commonly used genera for probiotics, namely *Lactobacillus* spp, *Bifidobacterium* spp, and *Enterococcus* spp are not the most abundant species in the large colon of the hosts. In fact, these species constitute less than 1% of the large intestinal microbiota in healthy horses.[6–8] In horses these bacteria are more abundant in the small compared with the large intestine. In younger animals (less than two months), they are more abundant independently of the gastrointestinal segment; however, still have low abundance when taken into relation with other bacteria.[6,8] The most abundant phylum in the equine gastrointestinal tract is Firmicutes, which contains the class of Clostridia. Important members of the Clostridia class, including *Ruminococcaceae* and *Lachnospiraceae*, have consistently been associated with gastrointestinal health in humans and animals, including horses.[9–11] Lactobacilli and bifidobacteria are not consistently associated with gastrointestinal health and might, therefore, have less influence on the gastrointestinal health of horses. To date, studies investigating effects of members of the Clostridia class are lacking. These might have better effects than currently available probiotic strains.

Studies in healthy animals are often used to assess the ability of a microorganism to survive transit in the gastrointestinal tract and persist after cessation of administration. Effective transit is defined as identification of probiotic microorganisms in fecal material, as determined by bacterial culture or polymerase chain reaction (PCR) assay. Identification of DNA with PCR does not imply viability, rather that a microorganism was present in the probiotic and recognizable DNA sequences survived gastrointestinal passage. Some investigators presume that colonization of the gastrointestinal tract with the probiotic strain is superior to mere effective transit because probiotics could act beyond the period of administration. In vitro studies have revealed, however, that effects of nonviable organism may be greater than the effects of viable organism. Therefore, lack of viability or persistence does not imply lack of effect.

Generally, host-specific strains are believed to be able to colonize the gastrointestinal tract of the indigenous host for longer periods of time. Colonization of the adult equine gastrointestinal tract with *Lactobacillus rhamnosus* LGG of human origin was shown to be poor.[12] After a 5-day course of probiotic administration at 3 different dosages (1×10^9, 1×10^{10}, and 5×10^{10} colony-forming units [CFUs]/g), fecal recovery in 21 adults was shown to be 71%, 29%, and 86% after 24 hours for each dose, respectively. After 48 hours, the probiotic was recovered from the feces of 14%, 14%, and 56% of the total dose administered, respectively, whereas 3 days after administration only one horse in each of the lower two dosage groups remained positive. Fecal recovery was longer in foals, where the probiotic could be recovered up to nine days after administration in some foals.[12] This suggests that the immature gastrointestinal flora of foals could facilitate probiotic survival.

As described previously, foals and adults showed a lack of dose response, making it difficult to determine an ideal dose to use.[12] Similar results were obtained when administering *Saccharomyces boulardii.*[13] After administration of 10×10^9 CFUs/g to 3 horses and 20×10^9 CFUs/g to 2 horses for 10 days, fecal samples were negative for *S boulardii* on day 20. On day five, all horses had viable *S boulardii* in their feces.[13] Similarly, *S cerevisiae* has been shown to survive but not colonize the ceca and colons of horses.[14–16] This indicates that any beneficial effect of probiotics might not continue beyond the period of administration, making long-term or repeated treatment a necessity.

To assess the effect of probiotics on the composition of the gastrointestinal microbiota, reduction of pathogens in vivo studies, in particular, have been conducted in other animal species. Knowledge regarding composition and function of the microbiota is not fully understood. For example, enteric pathogens, such as *Clostridium difficile* and *Salmonella*, can be isolated from feces of healthy animals[17]; therefore, presence of presumed pathogenic organisms does not necessarily cause disease and any attempt to reduce carriage might be ill advised. Recently, studies have shown that microbiota diversity and certain members of the Clostridia class, such as *Lachnospiraceae* and *Ruminococcaceae*, are consistently associated with gastrointestinal health.[8,10,18] Maintaining diversity and increasing the abundance of these Clostridia could be a potential target of probiotic development. In horses, to date only one study has assessed the effect of probiotics on the composition of the microbiota in a small number of foals. A significant effect was not seen.[19]

REGULATION OF COMMERCIAL PROBIOTICS AND QUALITY CONTROL

In North America (NA), most commercial probiotics are sold over the counter, without published efficacy or safety studies or ongoing quality control. In the United States, probiotics, also called *direct-fed microbials*, can be classified as a drug or as a dietary or feed supplement. If classified as a drug, the product needs to be approved and undergo quality control based on rules set forth by the Food and Drug Administration (FDA). Currently there are no approved probiotic products classified as drugs for horses in NA. If probiotics are classified as dietary or feed supplement, they fall under the category, “generally regarded as safe (GRAS),” and do not need to go through FDA drug-level approval. The FDA requires that supplements be labeled in a truthful and not misleading manner. The producers just provide an expert opinion on why the product should be considered GRAS and be approved by the FDA. The labels need to contain information to identify the feed additive, its concentration, and details on its safe and effective use. Claims that a feed additive can be used to cure, treat, or prevent disease named expressed or implied care are not allowed. The Center for Veterinary Medicine of the FDA, however, permits the use of meaningful health information; for example, “gastrointestinal health” claims on horse feed fall under this policy. Consequently, in NA, there are numerous probiotic products for use in horses on the market that can be obtained over the counter and claim to benefit the horse in various ways. Peer-reviewed published studies proving the efficacy of these products, however, are limited or in most cases lacking.

Even though the FDA regulates the labeling of probiotic products, many commercial veterinary and human probiotic preparations are not accurately represented by label claims. Studies evaluating labels of detailed contents of human and veterinary products showed that only 43% of human products and 8% of veterinary products from Canada were adequately labeled. There were inadequate descriptions of the bacterial content, including missing names, unspecified strains, nonexisting names, potentially pathogenic genera (eg, *Staphylococcus*) and outdated names.[20,21] Quality control of the active ingredient of commercial probiotics is also poor. Only 15% of veterinary and human probiotics contained the specified organism at the label claimed concentrations. Some products were missing organisms entirely or contained too little or too much of an active ingredient (0%–215% of the claimed amounts). All veterinary products contained less than 2% of the listed concentration of bacteria.[22] The effect of probiotics in a clinical setting might, therefore, not be predictable due to inadequate and inconsistent content of commercial probiotic formulations. Currently available published or claimed results of studies are likely also affected by this poor quality control. Commercial products used in research studies and evaluated as part of such, contained up to 100 times less active ingredient than what was claimed on the label.[23] The lack of effect seen in this particular study could be due to the inadequate content of the product rather than actual lack of biological effect. Even when using specifically designed (self-made) formulations, the content can be inadequate due to storage or production issues, and should be evaluated.[19]

MECHANISMS OF ACTION OF PROBIOTICS

Proposed mechanisms for the effect of probiotics include modulation of the host immune system, production of antimicrobial substances, inhibition or inactivation of bacterial toxins, displacement of pathogenic microorganisms (competitive exclusion), improvement in micronutrient absorption, and improvement in epithelial barrier function (**Fig. 1**).

The local intestinal and general immune system, including innate and adaptive responses are influenced by probiotics.[24] Conserved recognition receptors of the intestinal epithelial cells (IECs) and gut-associated immune cells recognize probiotics, and their metabolites, process them and evoke a signaling cascade. The effects include maintenance and fortification of the intestinal barrier by maintaining tight junctions, production of mucus, and survival and growth of IECs. This results in a stronger mucosal barrier against invading pathogens. Furthermore, the gut associated lymphoid follicles are presented with components of the probiotic strains by antigen presenting cells and induce differentiating of B cells into plasma cells. As a result, IgA, which is important for mucosal immunity, is released into the intestinal lumen

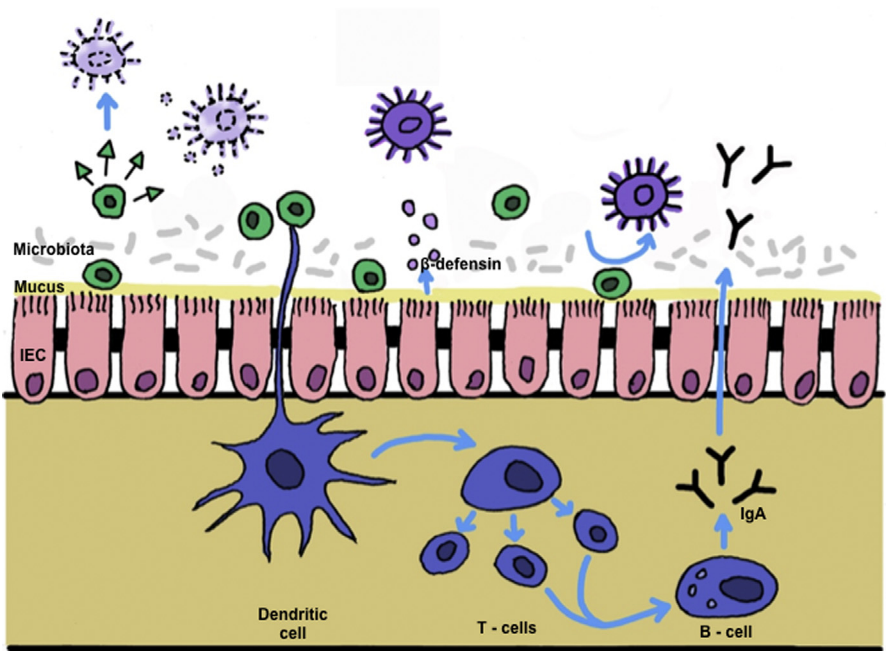

Fig. 1. Mechanism of action of probiotics. Green, probiotic; purple, pathogen.

by plasma cells.[25] The IgA and β-defensin, produced and released by IECs results in suppression of growth of pathogens as well as systemic and local anti-inflammatory effects.[24] Probiotics also modify the cytokine production by IECs and cells of the innate immune system, such as macrophages and dendritic cells, and therefore show an anti-inflammatory effect.[26] Systemically, probiotics can influence immunoglobulin production by altering systemic immunoglobulin isotope profiles.[27] Most of these studies assessing the effects of probiotics on the host have been conducted using human or laboratory animal cell lines, but to date no similar studies for horses have been published. Given the close conservation of the immune system across species, it is likely that similar effects do occur in horses.

Some probiotic strains produce antimicrobial metabolites in large quantities. These include fatty acids, lactic acid, and acetic acid. Other antimicrobial substances, such as formic acids, free fatty acids, ammonia, hydrogen peroxide, diacetyl, bacteriolytic enzymes, bacteriocins, antibiotics, and several undefined substances, are produced in much smaller amounts.[28]

Competitive exclusion refers to the ability of probiotic strains to decrease the amount of pathogens present in the intestinal lumen and their capacity to adhere to epithelial cells. Probiotic strains adhere to epithelial cells, block receptors and increase mucin production. Subsequently pathogens can no longer adhere or gain entrance into epithelial cells.[29] Additionally, probiotic strains occupy ecological niches and compete for nutrients, thus making it more difficult for pathogenic bacteria to survive in the gastrointestinal lumen.

Toxins are important and well-described virulence factors for enteropathogenic bacteria. Probiotics have been shown to inactivate toxins, reduce toxin production, and sometimes render toxigenic enteropathogens nonpathogenic.[30] The antitoxin effect of some probiotics may be beneficial in positively managing infectious diarrhea.

SAFETY OF PROBIOTICS

Adverse effects of probiotic administration have been rarely reported. In humans, the few reports available describe extraintestinal infections rather than enteric disease.[31]

Fungaemia due to treatment with *S boulardii* has been reported in human neonates.[32] Whether this could be a problem in horses is unknown, because studies with *S boulardii* in equine neonates have not been performed.[33]

Probiotics typically are used in individuals with enteric disease and adverse enteric consequences might be hard to distinguish from the primary disease present. It is reasonable to assume that the incidence of adverse events is very low, consistent with the GRAS classification of probiotics. In adult horses, there are no published reports of enteric disease after probiotic administration.[12,34] Doses are generally extrapolated from human recommendations and adjusted by weight.[13,35] Even administration of up to three times the manufacturers' recommended doses to healthy horses did not result in any adverse effects.[36] The effect of probiotics in horses with enteric disease might differ from the effect in healthy horses, but no adverse clinical effects have so far been reported in horses with gastrointestinal diseases either.[13,33,36] Most investigators, therefore, consider probiotics safe to be used in healthy and diseased adult horses.

The safety of probiotic use in foals has to be assessed independently of adults because there are major differences in immune system function and gastrointestinal microbiota composition between adults and foals.[37] Particularly, during the first month, the diversity is significantly decreased compared with older foals.[38] Although several published studies demonstrate safety of commercially available and self-made probiotics in foals,[34,39] there are also reports on adverse enteric effects.[19,35] In both cited studies a self-made probiotic was evaluated as a preventative measure for neonatal diarrhea in a placebo-controlled trial. The treatment group showed increased incidence of diarrhea and need for veterinary intervention in both studies.[35,40] Although it is unclear why these foals developed diarrhea, the immature microbiota of the foals could have allowed overgrowth of lactic acid bacteria, resulting in osmotic imbalances and diarrhea. Alternatively, the probiotics could have changed the microbiota to allow for pathogen adhesion to epithelial cells. The microbiota remains relatively stable in foals after two months of life but compared with adult horses differences are still present[38]; therefore, probiotic administration to foals more than two months old is less likely to result in severe side effects. Probiotics should be used with caution in foals, and safety studies should be performed before a product is administered.

CURRENT EVIDENCE FOR PROBIOTIC EFFICACY IN TREATMENT OR PREVENTION OF EQUINE GASTROINTESTINAL DISEASE

Probiotics have been administered to adult horses with acute enteropathies and to decrease Salmonella shedding. Although some studies have shown beneficial effects of probiotics, other studies could not corroborate these results (**Fig. 2**).[13,23,33,36,41–43] Overall, few studies are available, and these cannot be compared easily due to differences in study design, outcome parameters, and formulations used. Often one outcome parameter was influenced positively, such as duration of diarrhea; however, no effect on mortality or duration of hospitalization was seen. The more outcome parameters studied, the more likely significant effects are observed. Consequently, to date, the overall evidence is weak. The effect of a probiotic in a specific clinical context is likely unique to that context. The effect of a probiotic in horses with a specific disease cannot be extrapolated from results of studies in healthy horses or on horses with another disease. Also, extrapolation from humans or other species should not be done. Because clinical context is important, studies on the use of probiotics should ideally define the study population (healthy horses, horses with a specific disease, and so forth) and fully describe the probiotic (exact strain, dose, dosage regimen, and manufacturing details).

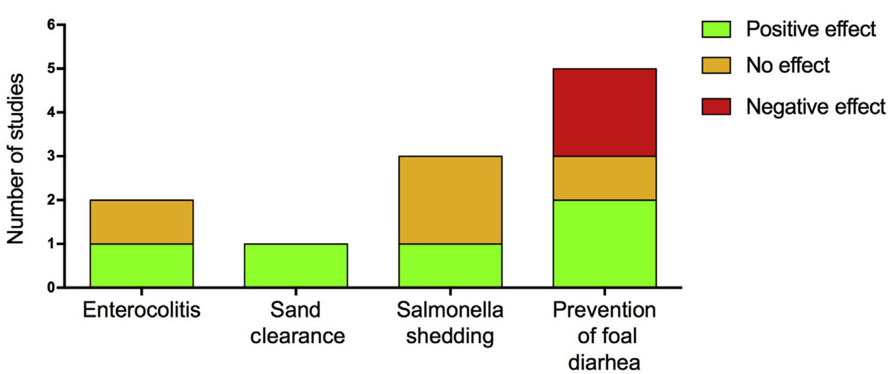

Fig. 2. Summary of effects of probiotics on preventing or treating gastrointestinal disease in horses.

Use of Probiotics for Treatment of Acute Enterocolitis in Adult Horses

S boulardii has been used with equivocal results. In one randomized, blinded, placebo-controlled clinical trial, horses receiving the probiotic had a shorter duration of diarrhea and watery diarrhea but not loose feces.[13] Despite this potential positive effect, the results of this study must be interpreted with caution, because there was no difference between the groups in relation to outcome, duration of hospitalization, and recurrence of diarrhea. In addition to the confounding factor of additional treatments, the number of cases was low, with only seven horses in each group.[13] In the second randomized placebo-controlled clinical trial involving 12 horses, no significant differences were observed between groups for occurrence of normal fecal consistency or cessation of watery diarrhea.[33] Also, days to improvement in attitude, resolution of leukopenia, appetite, normalization of heart rate, respiratory rate, and temperature, length of stay in hospital, survival to discharge, and occurrence of secondary complications were not different between groups.[33] Although this study was influenced by fewer confounding factors and evaluated more clinical variables compared with the previously cited study,[13] the number of cases was low and adjunct treatment was variable among horses, making interpretation of the results more difficult. Although both studies investigated the same probiotic, they are difficult to compare due to a heterogeneous horse population and different inclusion and outcome criteria. In summary, the evidence for an effect of *S boulardii* as an adjunctive treatment of enterocolitis in horses is weak. Additional probiotic strains have not been studied in adult horses with acute enterocolitis. Overall studies in horses provided weak evidence for the use of probiotics.

Salmonella Infection and Shedding

In horses, the effect of probiotics on *Salmonella* shedding has been investigated but results have been disappointing to date. No differences in *Salmonella* shedding rates, prevalence of postoperative diarrhea, leukopenia, length of antimicrobial therapy, and length of hospitalization were found between groups.[36,41] The overall number of horses shedding *Salmonella* was small, fecal samples were taken at irregular, arbitrary intervals, and, given that *Salmonella* shedding can be intermittent, some cases might have been missed.[23,44] These limitations could have influenced the results of these study. In contrast, administration of a probiotic decreased the incidence of *Salmonella* shedding by 65% in another study, but the difference was not significant (P = .19) due to low power of the study.[23] In other species, it is known that the effect of probiotics depends on the agent studied, and so far few probiotics have been evaluated in horses. Additional studies are necessary before excluding the beneficial effects of probiotics on *Salmonella* shedding in horses. There is currently little evidence supporting the use of probiotics to decrease *Salmonella* shedding or salmonellosis in horses.

Current Evidence for use of Probiotics in Foals

Probiotic administration has been studied as a preventative measure for foal diarrhea in several studies with variable results (see **Fig. 2**).[35,39,40,45,46] Probiotic administration was associated with a significantly higher incidence of diarrhea, presence of clinical signs (lethargy, fever, and anorexia colic) and the need for veterinary examination and treatment in two studies.[35,40] In another study, foals in the probiotic group showed statistically significant larger weight gain after treatment and a significantly lower incidence of diarrhea.[39] These effects, however, were only significant at specific time points. It is unlikely that the diarrhea was clinically important as evidenced by a lack of difference in the need for medical intervention between the 2 groups. In a fourth study on prevention of neonatal diarrhea in foals, a reduction of diarrhea incidence by 60% could be achieved with probiotic administration.[45] Although this result seems promising, there were several limitations to this study, including unequal treatment groups and inconsistent monitoring (every two weeks), which could have resulted in oversight of many diarrhea episodes. Additionally it is unclear how often foals were treated with the probiotic and the study was not blinded. In another study, *Bacillus cereus* supplementation did not have an effect on incidence of diarrhea during the first 58 days of life in a study in 25 foals, irrespective of the dose used.[46] These studies cannot be directly compared because different products were used. In summary, every probiotic product that is used in neonatal foals should be evaluated for safety and efficacy before administration. Larger-scale controlled studies of different strains and products are necessary before conclusions can be drawn on the clinical efficacy of probiotics in foals with diarrhea.

FECAL MICROBIAL TRANSPLANTATION

All current probiotics consist of one or a few strains that comprise a minor component of the intestinal microbiota. Therefore, they might have limited ability to influence the entire gastrointestinal microbiota. Scientists and clinicians are now evaluating the other end of the probiotic complexity spectrum, fecal transplants. Fecal transplants consist of an intact, highly complex microbial community composed by thousands of species. Fecal microbial transplantation constitutes the transfer a fecal suspension from a healthy donor into the bowel of the recipient. Fecal microbial transplantation has been shown to be highly effective in treating recurrent *Clostridium difficile* infections in humans. Although there are no published studies or abstracts in horses, anecdotal reports suggest that this form of therapy also might be effective in horses with acute colitis or chronic diarrhea.[47,48]

SUMMARY

Although probiotics have shown promise in treatment of selected diseases in humans, the evidence in horses is weak. For any given disease, only few probiotic organisms have been evaluated so far and most studies were underpowered or confounded. On the basis of examination of existing data, no specific product can be recommended for use. The aim of developing the one probiotic to aid in prevention or treatment of all diseases is unrealistic. The choice and combination of strains for a therapeutic formulation needs to be specific for each disease and should be based on the in vitro properties of the strains and tested in clinical placebo controlled randomized trials. Based on lack of regulation regarding quality control of commercial products, use of over-the-counter products is questionable, particularly in the absence of scientific information on safety and clinical efficacy. Probiotics likely have a different effect in foals because the gastrointestinal microbiota transitions to the adult microbiota during the first months of life. Adverse effects have been reported in foals and probiotics should be evaluated for safety before use in foals. Despite all these limitations, probiotics generally are regarded as safe, and easy to administer. Therefore, additional research is warranted to test possible applications in equine veterinary practice. Exploiting new knowledge of the composition of the equine microbiota, the focus of probiotic research should shift from currently used agents to species that are abundant in the intestinal microbiota of the horse. By combining microbiota research, research on fecal microbial transplantation and probiotic research, a designer probiotic containing all beneficial microbes could eventually be developed.

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